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14. ABSTRACT
TTNatural and semisynthetic gangliosides protect neurons from toxin-induced cell death and salvage neurons after toxin exposure. The hydrophilic property of gangliosides restricts their blood-brain barrier (BBB) permeability, which hinders their use as neuroprotective agents. Gangliosides semisynthetic derivatives with improved cytoprotective properties and BBB permeability can be produced. Even with gangliosides' great therapeutic promise, no study has examined ganglioside functional group derivatives that would provide cytoprotection AND effectively cross the BBB; information that would provide a basis for future studies of neuroprotective mechanisms. This study examined the ability of ganglioside derivatives to be cytoprotective in in vitro models using the dopaminergic neurotoxin, 1-methyl-4-phenylpyridinium (MPP+) and the SH-SY5Y cell line. Derivatives determined to have therapeutic potential were to be tested in vitro for their ability to cross a brain capillary endothelial cell culture model of the BBB. Finally, derivatives that were both cytoprotective and that effectively crossed the in vitro BBB model were to be tested in vivo for their ability to neuroprotect dopaminergic neurons in both chronic and acute neurotoxicity models using the MPP+ precursor, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The hypothesis is that changes in ganglioside ceramide and/or oligosaccharide functional groups can improve neuroprotection through changes in cytoprotection and BBB transcytosis.

15. SUBJECT TERMS
Parkinson's disease, MPTP, MPP+, GM1, gangliosides, ganglioside derivatives, SH-SY5Y cells

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INTRODUCTION

Note in explanation: This report is comprised of the annual report for the year September 2004 through September 2006. This combined report is with permission of the Department of Defense and is so constructed because the Principal Investigator (PI) was seriously ill for the majority of this time interval. Because the illness, therapy, and recovery periods were so extensive, the PI is no longer a research investigator and has resigned his position at the University of Massachusetts Medical School.

Gangliosides are naturally occurring acidic glycolipids that are enriched in neural tissue. They broadly protect different types of neurons *in vitro* (1;2) and preserve diverse neuronal (3-5) and behavioral (6;6) functions from a variety of neurotoxic insults *in vivo*. The fact that gangliosides appear to protect neurons from various neurotoxic insults by several different mechanisms makes them ideal candidates for, or models upon which to derive, therapeutic agents for Parkinson's disease and other neurodegenerative diseases with potentially diverse pathological mechanisms. Gangliosides are heterogeneous compounds differing in the composition of their lipophilic and hydrophilic moieties. It has been shown with the monosialoganglioside GM1 that the cytoprotective effects and blood-brain barrier permeance differ among the various molecular structures. This implies that the protective effects of gangliosides can be optimized by semisynthetically varying their lipophilic and hydrophilic moieties. Many neurotoxins work through mechanisms that involve mitochondrial electron transport chain inhibition and oxidative stress, proteasomal inhibition, or excitotoxicity. Gangliosides have been shown to be protective against a range of neurotoxins in various models. For example, GM1 protects dopaminergic neurons from MPTP (7;8) and MPP^+ (9) (a mitochondrial complex I inhibitor) even when administered after neurotoxin exposure (10). Gangliosides appear to be protective through several mechanisms. These minimally include their effects on growth factor production and receptor activation (11), signal transduction (12), membrane order and raft formation (13), calcium homeostasis (14), membrane trafficking, excitatory processes (15), altered enzyme activity (such as superoxide dismutase (16), catalase (17) or phosphatidylinositol 3-kinase (2)) and modified protein kinase C translocation (3). It is, of course, important to ultimately understand the mechanism of ganglioside neuroprotection. There is, however, neither an adequate understanding of ganglioside functional groups and required structure necessary to produce significant neuroprotection, nor a sufficient knowledge of the functional group alterations that allow ganglioside derivatives to effectively cross the BBB while maintaining cytoprotective ability. In fact, investigations into ganglioside protective mechanisms are daunting endeavors because of the apparent diverse modes of action. Thus, one approach to defining structure-function relationships in ganglioside neuroprotection is to first describe the structural requirements for neuroprotection and then to define the mechanisms of action.

One problem in using gangliosides for therapeutic purposes is that they are ionic and hydrophilic. This prevents them from efficiently crossing the BBB. An approach to overcoming the BBB limitation is to make gangliosides more lipophilic by derivatizing the parent compounds (semisynthetic gangliosides), and, presumably, making them better able to cross the BBB. The internal ester (lactone) of GM1 is chemically neutral (and, therefore, more lipophilic) and it is also neuroprotective *in vivo*, often more potently than the parent GM1. (19), (20), (21), a (22). The apparent improvement, compared to the parent GM1, in the *in vivo* neuroprotection by ganglioside derivatives such as the internal ester or LIGA 20, may be due to either or both improved neuroprotective properties, and improved ability to cross the BBB.

This study examines ganglioside functional group derivatives that provide cytoprotection AND effectively cross the BBB; information that will provide a basis for future studies of neuroprotective mechanisms. This research studies the ability of ganglioside derivatives to be cytoprotective in *in vitro* models using the dopaminergic neurotoxin, 1-methyl-4-phenylpyridinium (MPP^+) and the SH-SY5Y human neuroblastoma cell line. Derivatives determined to have therapeutic potential are tested *in vitro* for their ability to cross a brain capillary endothelial cell culture model of the BBB. Derivatives that are both cytoprotective and that effectively cross the *in vitro* BBB model will be tested *in vivo* for their ability to neuroprotect dopaminergic neurons in both chronic and acute neurotoxicity models using the MPP^+ precursor, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). This research studies the hypothesis that "changes in ganglioside ceramide and/or oligosaccharide functional groups will improve neuroprotection through changes in cytoprotection and BBB transcytosis." This research will provide a basis to improve ganglioside neuroprotection in neurodegenerative diseases, e.g., Parkinson's disease and neurotoxin exposure.

BODY

Report

This proposal consists of 4 specific objectives in the **Statement of Work**.

Statement of Work, Objective 1- Semisynthetic ganglioside derivatives will be synthesized from gangliosides isolated and characterized in the P.I.'s laboratory. These will include derivatives of the ceramide fatty acids, oligosaccharide functional groups, including internal esters, asialo, and reduced carboxylic acid (gangliosidol), and combinations of ceramide and oligosaccharide derivatives. Syntheses will be performed as described in Methods. Semisynthetic ganglioside derivatives will be characterized by chemical and mass spectrometric techniques.

During the course of this project, over 10 gms of GM1 were isolated and characterized by chemical, chromatographic, and mass spectrometry techniques. The isolated GM1 was carefully characterized by chemical and instrumental analysis to insure purity, including the absence of peptides (Serma and Touchstone, 1974). The isolated GM1 was resorcinol and orcinol positive, yielded a negative response to ninhydrin, and was retained by anion exchange resins (Ledeen and Yu, 1982). High-performance thin-layer (HPTLC) chromatography provides R_f values comparable to known GM1 standard and showed no contaminating materials on examination with ninhydrin, resorcinol, orcinol, or charring sprays. Mass spectrometry analysis provided the expected spectra for pure natural GM1 (Figure 1, Panel A).

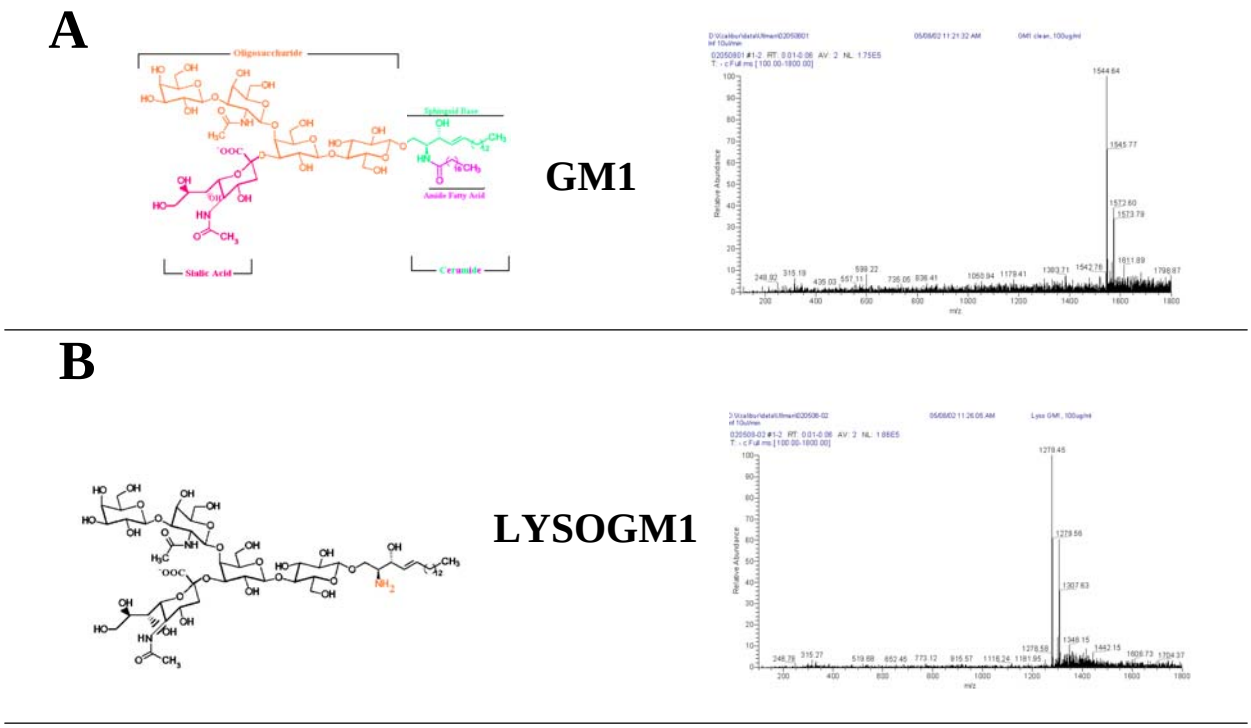

Figure 1. Structures and mass spectra of GM1 (Panel A) and lysoGM1 (Panel B): starting materials for synthesis of derivatives

LysoGM1 was synthesized from the isolated GM1. The lysoGM1 was tested for cytoprotective properties and used as the starting material for the synthesis of ganglioside ceramide fatty acid derivatives. It wass produced by chemical reaction of GM1 with KOH in isopropanol (Sonnino et al., 1992) and characterized in the same fashion as GM1. The isolated and purified lysoGM1 was characterized to insure purity. The material was resorcinol and orcinol positive, and yielded a positive response to

ninhydrin. HPTLC provided R_f values comparable to known lysoGM1 standard (Matreya Inc. State College, PA) and showed no contaminating materials on examination with ninhydrin, resorcinol, orcinol, or charring sprays. Mass spectrometry analysis provided the expected spectra for pure lysoGM1 (Figure 1, Panel B). Other derivatives synthesized included LIGA 20 (dichloroacetylGM1), and the C2, C4, C8, C12, C14, C20, C24, and C26 fatty acid gangliosides. Low yields of the lysoGM1 and the presence of appreciable side products from the propanolysis initially slowed progres. Yields weree increased by altering the reaction conditions. Purification of lysoGM1 and the ganglioside derivatives was performed by high-performance liquid chromatography (HPLC). Up to 5 mg of glycoconjugate are placed on a 55 cm x 7 mm I.D. Iatrobead column (mean particle size 10μm) and separated with a flow rate of 1 ml/min and a linear 140 min mobile phase gradient of 82.4% solvent A (isopropanol:hexane:water 55/38/7, v/v/v) and 17.6% solvent B (isopropanol, hexane, water, 55/20/18, v/v/v) to 51.4% solvent A and 48.6% solvent B followed by and additional 40 min. isocratic plateau at the final gradient conditions. In the case of all derivatives, purification presented the hardest challenge. For the most part, these challenges were overcome by HPLC purification of the derivatives.

The yields of ganglioside derivatives was improved by the purchase of a chemical synthesizer that allowed better control of reaction conditions including the ability to maintain an inert gaseous atmosphere for the synthesis of lysoGM1, the precursor to the ceramide fatty acid derivatives. In addition, oligosaccharide derivatives of GM have been made including asialoGM1 and the GM1 internal ester.

One serious problem arose during the synthesis of ganglioside derivatives that resulted in the destruction of the total lysoGM1 supply that had been synthesized at one time in this project. LysoGM1, the starting material for all of the GM1 fatty acid derivatives is sensitive to acid hydrolysis and must be handled in a neutral or slightly alkaline condition. Chloroform purchased from a reputable source (Fisher Scientific) contained acid well beyond product specifications. This problem was not discovered for some time because the visual consequences manifested themselves identically to problems with chromatographic purification. This source of chloroform has been used in this laboratory for two decades with no previous problems. When replacement chloroform was ordered, the acidic condition still existed even though the company was aware of the problem and even though it had assured us the problem was resolved. Eventually, we negotiated with Fisher Scientific to obtain a fraction of compensation for the lost time, materials, and starting material.

After recognition of the source of lysoGM1 degradation, synthesis of the lysoGM1 reaction intermediate progressed relatively well and significant quantities were either made in the laboratory or

provided gratis by Fisher Scientific. Ganglioside derivatives consisting of C2, C4, C8, C12, C14, C20, C24, and C26 were synthesized and characterized (Table 1).

Compound	Resorcinol	Orcinol	Ninhydrin	Retained By Ion Exchange	$[M-H]^-$ C18:0-d18:1	$[M-H]^-$ C18:0-d20:1	Parent of Mass Ion 290 (Sialic Acid)
GM1	+	+	-	yes	1544	1572	Yes
GM1 inner ester	+	+	-	no	1526	1554	not tested
lysoGM1	+	+	+	not tested	1261	1289	not tested
N-acetyl GM1 (LIGA4, C2)	+	+	-	yes	1320	1348	not tested
N-dichloroacetylGM1 (LIGA20, dichloroC2)	+	+	-	not tested	1388	1416	not tested
GM1 gangliosidol	+(faint)	+	-	no	1530	1558	not tested
N-acetyl GM1 inner ester	+	+	-	no	1302	1330	not tested
AsialoGM1	-	+	-	no	1253	1281	not applicable
N-butyl GM1 (C4)	+	+	-	yes	1348	1376	Yes
N-capryl GM1 (C8)	+	+	-	yes	1404	1432	Yes
N-myristyl GM1 (C14)	+	+	-	yes	1488	1516	Yes
N-arachidyl GM1 (C20)	+	+	-	yes	1572	1600	Yes
N-lignoceryl GM1 (C24)	+	+	-	yes	1628	1656	Yes
N-cerotyl GM1 (C26)	+	+	-	yes	1656	1684	Yes

Table 1. Characterization of gangliosides and ganglioside derivatives. C18:0, 18 carbon saturated fatty acid; d18:1, sphingosine (18 carbons, 1 double bond); d20:1,eicosasphingosine (20 carbons, 1 double bond).

Of the many procedures available for the synthesis of fatty acid molecular species of GM1 we found the procedure of Lapidot et al. to be the most effective {7639}. Recently, there has been published a procedure for the large scale enzymatic synthesis of lysoGM1 using sphingolipid ceramide deacylase {7845}. This procedure is recommended for future preparations of lysoGM1 as it produces fewer side-products and eliminates exposure to chloroform.

The derivatives have been transferred to the laboratory of Dr. Wayne Lencer, Childrens Hospital, Boston, MA. Dr. Lencer was a collaborator and will utilize the compounds to further investigate membrane and toxin trafficking to subcellular organelles.

Statement of Work, Object 2 - Semisynthetic derivatives that specifically retain or improve the cytoprotective properties of the parent compound will be determined by testing them *in vitro* using an $MPP^{\pm}$ model of neurotoxicity in SH-SY5Y cells. Cytoprotection will be evaluated by use of the MTT assay, trypan blue exclusion with cell counting, and neurochemical analysis of DA, DOPAC, and HVA.

In a previous report it was indicated that lysoGM1 and GM1 showed roughly equivalent cytoprotection in the SH-SY5Y cell culture system. The difference between GM1 and lysoGM1 is that lysoGM1 has a free amine group where GM1 has an amide-linked fatty acid. Because of this difference in structure it

was felt that the two compounds could have different cytoprotective mechanisms so that their protective effects might be additive. A test of this possibility revealed that the protection was not additive and the commonality of the mechanism(s) of action of these two compounds remains unknown.

The ability of GM1 (no preincubation) to protect RA-differentiated SH-SY5Y cells from MPP^+ toxicity was determined with the MTT assay 48 hr after toxin exposure (Figure 2). The MTT assay provides an evaluation of mitochondrial status. Experiments consisted of at least 4 wells per data point and the experiment was performed in triplicate.

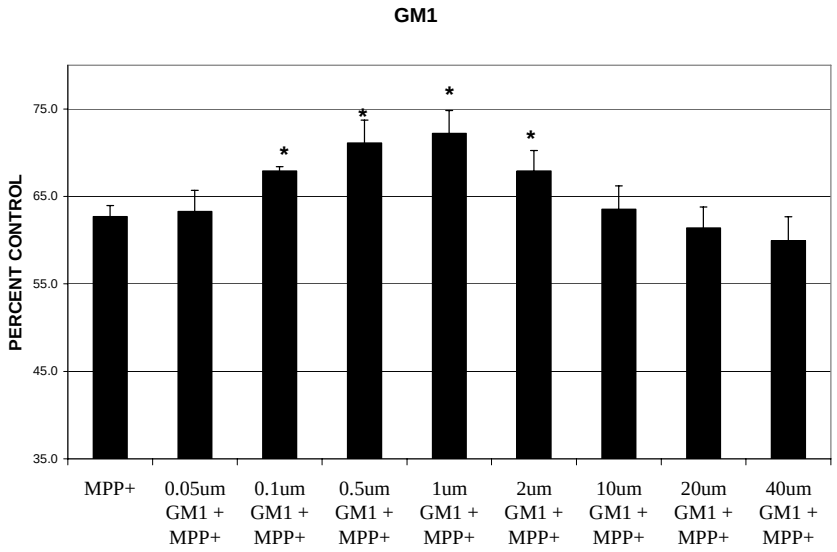

Figure 2. GM1 cytoprotection. Increasing concentrations of GM1 partially protected SH-SY5Y cells from MPP^+ (1mM) toxicity. Concentrations at or above 2uM did not provide protection.
Values represent the mean $\pm$ SEM of 4 wells
* $p<0.05$

GM1 provided partial protection against MPP^+ toxicity with maximal protection at 1 uM. Higher GM1 concentrations were not protective. The mechanism of this loss is unknown but may be due to the detergent effect of the amphipathic GM1. This phenomenon was seen with all of the derivatives tested and warrants further investigation. Incubation of GM1 alone with SH-SY5Y cells under these conditions did not significantly alter cell viability compared to no GM1 controls.

The literature indicates that gangliosides may require a preincubation period prior to addition of the toxin. We tested the need for preincubation with the parent GM1. The SH-SY5Y cells were tested at 48 h after MPP+ exposure. The cells were preincubated with GM1 for 2 hr before toxin addition and

the amount of protection was compared to that with no preincubation and to the presence of toxin alone (Figure 3). Preincubation did not improve protection. Shorter (0.25 hr and 0.5 hr) and longer (4 hr and 6 hr) incubation times were also examined in a separate experiment with no improvement in cytoprotection compared to no preincubation controls. Therefore, for the MPP^+ experiments, no preincubation of GM1 or the derivatives was used.

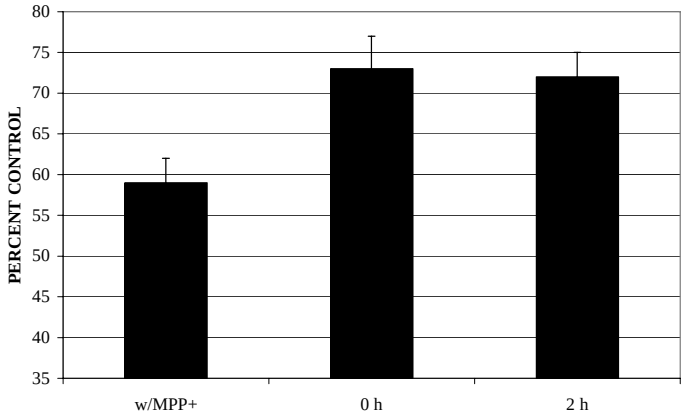

Figure 3. Preincubation of the GM1 is not required for cytoprotection.
Values are mean ± SEM
* different from MPP^+ alone, $p<0.01$, N = 6 wells

Although preincubation is not required for GM1 in this experimental paradigm, preincubation experiments to investigate optimal preincubation intervals for each new derivative to be tested are included in the experimental design.

Subsequent to establishing conditions for GM1, several GM1 derivatives have been tested *in vitro* using the MTT assay. For each derivative tested, dose response and preincubation curves were run. Cell culture plates (48-well) were utilized and each data point consisted of either 4 or 6 wells depending on the experimental design and each experiment was run in duplicate or triplicate with the exception of the GM1 inner ester which has been run only once to this time. For brevity, comparisons are presented at the derivative concentration with maximal cytoprotection (Figure 4). All GM1 derivatives were cytoprotective with the exception of the asialo derivative, implying that the sialic acid is an important constituent for cytoprotection. LysoGM1, N-butylGM1, and N-myristylGM1 cytoprotect comparable to GM1 but their effective concentrations are significantly less than that for GM1 (lysoGM1 0.1 µM; butylGM1 0.05 uM; N-myristylGM1 0.04 uM). LysoGM1 (no ceramide fatty acid) was unexpectedly protective. This could imply that the fatty acid is not important in the cytoprotective mechanism or that the cytoprotective mechanism is different for lysoGM1.

The likelihood of different mechanisms of action is supported by the fact that different chain length ceramide fatty acid gangliosides differentially regulate growth factor signals (23). Also, the ceramide fatty acid is apparently important in the determination of BBB permeance. Experiments with combinations of derivatives will be tested for any additive effects of separate mechanisms. The protection afforded by the GM1 internal ester appears to be the most potent. Internal esters of the other derivatives are being prepared. Considerations for *in vivo* testing will include blood-brain barrier

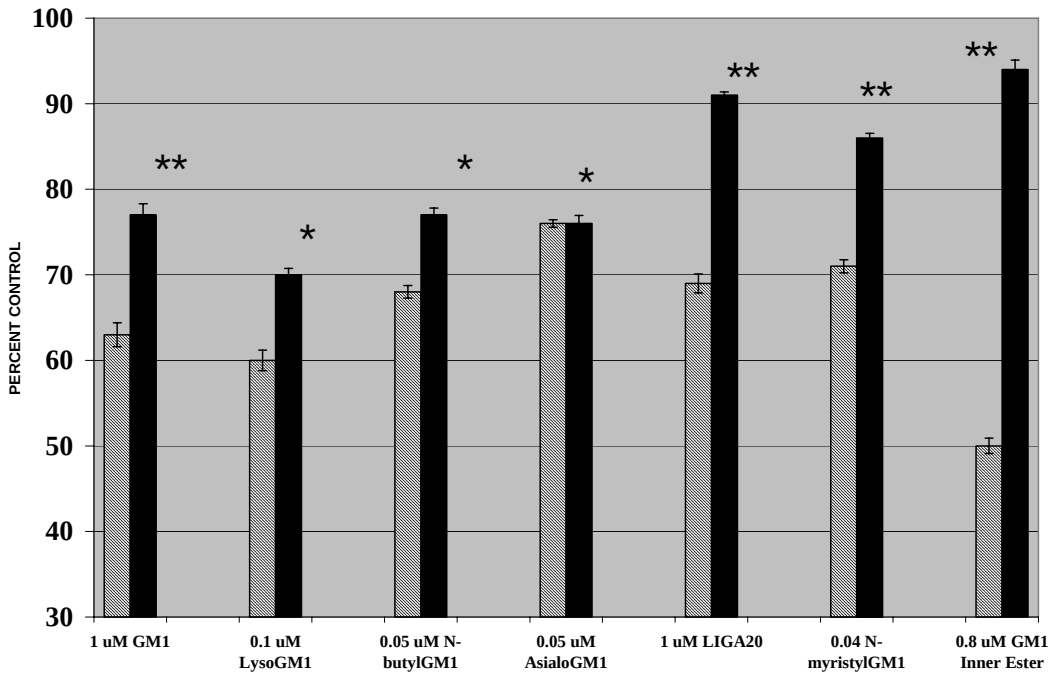

Figure 4. Comparisons of GM1 derivative cytoprotection. Values are expressed as percent control ± SEM. Stripped columns are percent control of MPP^+-treated RA-differentiated SH-SY5Y cells. Filled columns are percent control ± SEM of MPP^+-treated cells in the presence of GM1 or GM1 derivative. Differences between MPP^+ with or without GM1 or GM1 derivatives are significant except for asialoGM1.
*p<0.05, **p,0.01

permeance and effective concentration rankings.

Additional experiments have been performed (in collaboration with Dr. Kelly Conn, Dr. John Wells, Dr. Richard Fine at the VA Hospital, Bedford, MA) to further establish the mechanism of MPP^+ toxicity, which will help in the delineation of mechanisms by which ganglioside derivatives are

cytoprotective. To further define MPP^+ toxic mechanisms and Parkinson's disease neuronal degeneration, RT-PCR analysis was used to characterize the transcriptional response of retinoic acid-differentiated SH-SY5Y cells to MPP^+ exposure. We have shown that the protein disulfide isomerase (PDI) family member pancreatic protein disulfide isomerase (PDIp), previously considered exclusively expressed in pancreatic tissue, is uniquely upregulated among PDI family members within 24 hours following exposure of retinoic acid-differentiated SH-SY5Y cells to 1 mM MPP^+ or to 10uM of the proteasomal inhibitor lactacystin. Further, lactacystin treatment increases clusterin expression. Clusterin (apolipoprotein J) is a highly conserved, multifunctional vertebrate glycoprotein. Several isoforms exist and the predominant secreted isoform (sCLU) is associated with cell death and binds a variety of partly unfolded, stressed proteins including those associated with Lewy bodies in Parkinson's disease. The development of familial and sporadic Parkinson's disease has been associated with the ubiquitin-proteasome system dysfunction and aberrant protein degradation. These findings on altered gene expression in experimental models of Parkinson's disease provide insight into possible protective mechanisms.

In a collaborative study with Dr. Wayne Lencer and Dr. Anne wolf, we have been looking at exogenous ganglioside trafficking as suggested by a reviewer of the previous report. Initial studies have been performed in a glioma cell line. Preliminary results indicate that the shorter chain ganglioside (N-butylGM1) associates more rapidly with the plasma membrane than does the parent GM1 but that the parent GM1 is a more effective receptor for cholera toxin subunit than the butyl derivative. These experiments are being repeated and the intracellular target for the bound cholera toxin is now under investigation. When conditions are well established, the uptake kinetics and intracellular trafficking will be examined in the SH-SY5Y cells.

Statement of Work, Objective 3, Effective cytoprotective semisynthetic ganglioside derivatives that effectively cross a brain capillary endothelial cell model of the blood-brain barrier (BBB) will be determined. Model BBB transcytosis will be assessed by liquid scintillation counting of radiolabeled derivatives in aliquots taken from the lower wells of Transwell cell culture plates.

An in vitro model of the blood-brain barrier was re-established in our laboratory utilizing a slight modification of a previously published cell culture system [11]. When isolated bovine brain capillary endothelial cells were 90% confluent they were plated on Transwell inserts that were previously coated with Fibronectin-like polymer. The cells were plated and then grown in 50% astrocyte conditioned medium and 50% Bovine Insert Medium (DMEMF12/N2 supplement). The cells were seeded at a density of $5x10^4$, and grown to form tight junctions in the presence of a phosphodiesterase inhibitor (Ro-20-1724) and 8-(4-chlorophenylthio)-cAMP [11]. Tight junction integrity was estimated by using a FITC dye (4kDa, 1 mg/mL, 250 uL to top chamber), in duplicate.

Samples (50 uL) were withdrawn from the lower chamber at 0, 10, 20, 30, 55, and 70 minutes (Figure 5.). Each time, the lower chamber was replenished with 50 uL of media and the dilution was accounted for in the final calculations. MDCK cells were run as tight junction controls. Withdrawn samples were placed in an opaque black back 96 well plate and wrapped in aluminum foil until read on the fluorometer.

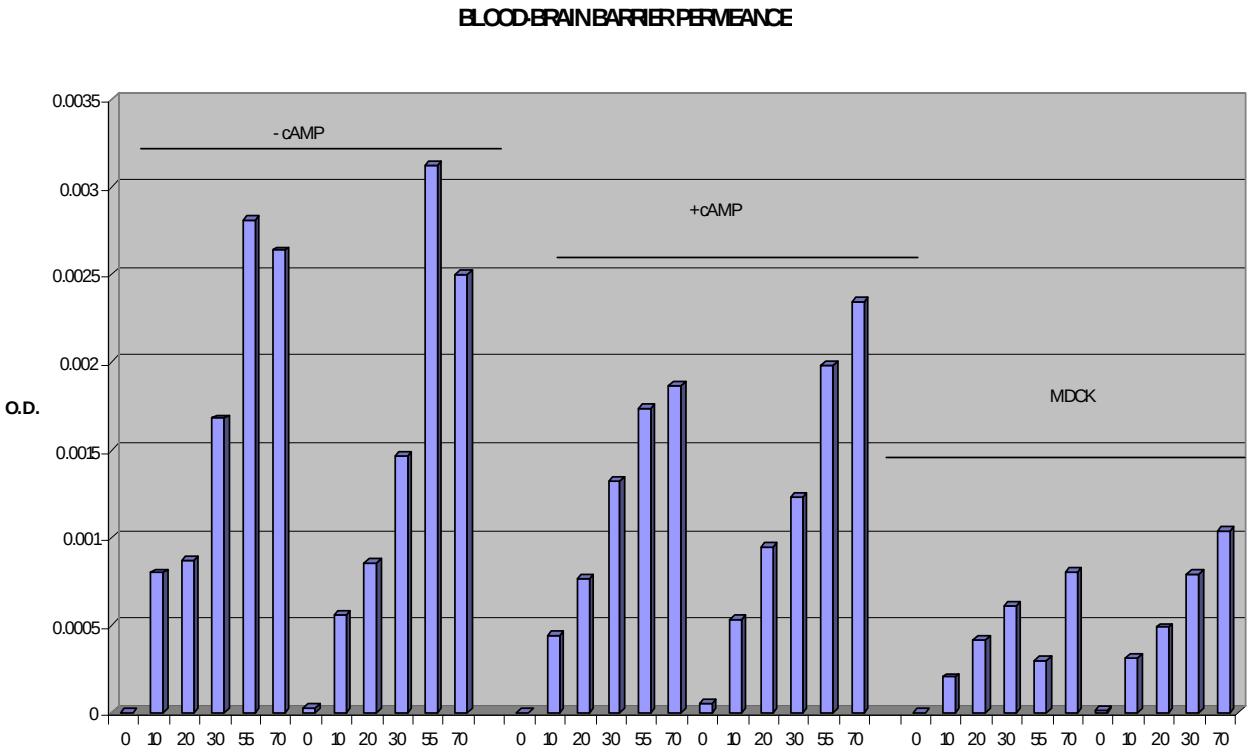

Figure 5. FITC fluorescence after incubation with bovine capillary endothelial cells that were incubated without (-cAMP), or with (+cAMP) a phosphodiesterase inhibitor (Ro-20-1724) and 8-(4-chlorophenylthio)-cAMP (to form tight junctions) or with MDCK cells as a positive tight junction control. the phosphodiesterase inhibitor (Ro-20-1724) and 8-(4-chlorophenylthio)-cAMP increased tight junction resistance to FITC transcytosis.

It was decided that the synthesis of radiolabeled ganglioside derivatives would be too time consuming, expensive, and would require too much of the previously synthesized derivatives to be practical. Instead, it was decided to perform the BBB model analyses on unlabeled derivatives by utilizing a mass spectrometry procedure. We therefore developed a procedure for the analysis of gangliosides

from the BBB in vitro model. An experiment was performed as proof of principle that the procedure would be applicable to the BBB samples. Ganglioside GM1 (3 nmol) was added to the upper chamber cell culture medium of a Transwell and incubated for two hours. The media from the chambers was isolated and an equal volume of 0.2M KCl in methanol containing 6 nmol of C4-GM1 derivative (as an internal standard) was added to each sample. The samples were then added to C18 solid phase extraction (SPE) disks (ANSYS) and each column was washed free of media and salts with 400 uL of water and GM1 was isolated with 400 uL of methanol. The ganglioside samples were then analyzed by direct Electrospray Full MS and Precursor Ion MS/MS. Aqueous gangliosides samples run as standards were diluted with 1or 2 vols. of methanol and infused directly into the ion source of a Waters Quattro-II triple quadrupole mass spectrometer at 3 µl/min. Methanol samples fromSPE cartridges were diluted with an equal volume of water. The ESI source was operated in the negative ion mode with the capillary at 4 kV, the sample cone at 130 V, desolvation temperature at 200^{o} C and the source block at 80^{o} C. Full MS were acquired from m/z 500 – 1,800 and averaged for 2 min. Precursor ion scans of m/z 290 (N-acetylneurminic acid) were acquired from m/z 500 – 1,800 with CID energy of 85 V and argon pressure of 1 e^{-3} mBar to specifically detect gangliosides. C4-GM1 and GM1 had approximately 1:1 responses. This procedure can be used to determine ganglioside permeance in the BBB model system (Figure 6).

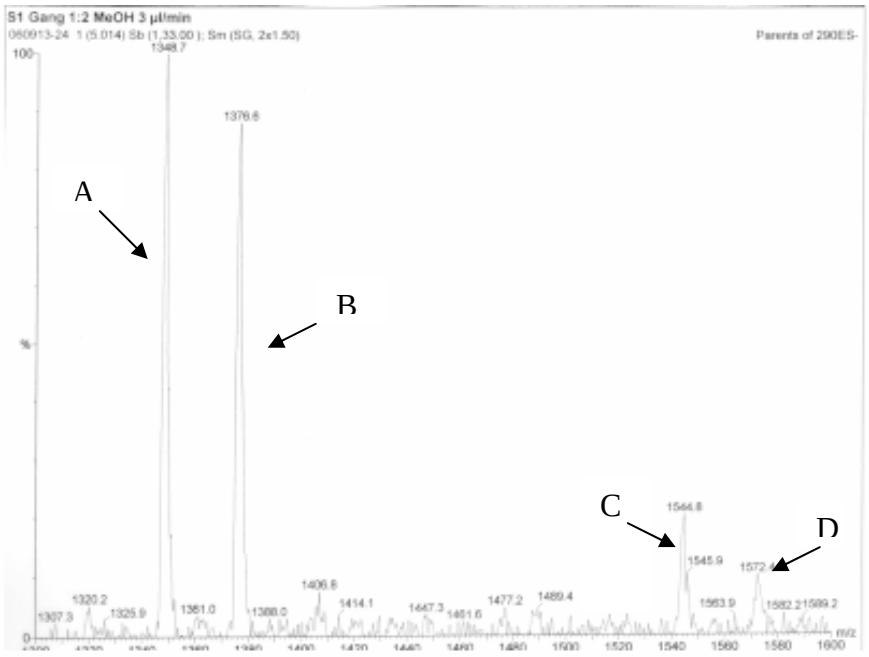

Figure 6. Mass spec identification of sphingosine isomers of C4-GM1 (A and B) and GM1 (C and D) isolated from the lower chamber of a Transwell. C4-GM1 was added as an internal standard. GM1 was added to the top chamber and incubated for two hours. Results indicate feasibility of performing BBB permeance by mass spectrometry thus avoiding the use of radiolabeled derivatives.

Statement of Work, Objective 4, Semisynthetic ganglioside derivatives that effectively protect neurons *in vivo* using chronic and acute MPTP administration models of neurotoxin insult will be determined by testing, in mice, those derivatives that both cytoprotect the SH-SY5Y cells from MPP^{+} toxicity *in vitro* AND that effectively cross the *in vitro* BBB model. The chronic and acute models represent apoptotic and necrotic cell death mechanisms, respectively. Neuroprotection will be evaluated by neurochemical analysis of dopamine (DA), 3,4-dihydroxyphenylacetic acid **(**DOPAC), and homovanillic acid (HVA), and by neuronal counts of Nissl substance, tyrosine hydroxylase (TH) and dopamine transporter (DAT) positive neurons of the substantia nigra pars compacta. Work on this objective was not completed.

KEY RESEARCH ACCOMPLISHMENTS

- Large quantities of Gm1 were isolated
- Synthesis of lysoGM1 was accomplished although difficulties were encountered to to acidic chloroform from the manufacturer
- Increasing concentrations of GM1 partially protects SH-SY5Y cells from MPP^{+} (1mM) toxicity.
- Concentrations at or above 2uM appeared to make the cells more vulnerable to MPP^{+} toxicity.
- Preincubation of the GM1 is not required for cytoprotection
- Ganglioside derivatives provide cytoprotection comparable to the parent GM1 but at lower concentrations
- GM1 protects against low potassium induced apoptotic cell death
- MPP^{+} or lactacystin upregulate the expression of PDIp
- Lactacystin increases clusterin expression
- Proof of principle that MS and MS^{n} can be used to determine the permeability of ganglioside derivatives in a bovine brain capillary endothelial cell model of the BBB.

REPORTABLE OUTCOMES

Manuscripts

Conn,K., Ullman MD, Larned MJ, Eisenhauer PB, Fine RE, Wells JM: cDNA Microarray Analysis of Changes in Gene Expression Associated with MPP^{+} Toxicity in SH-SY5Y Cells, *Neurochem. Res.* Neurochemical Research (2003) 28: 1873-1881

Conn KJ, Gao W, McKee A, Lan MS, Ullman MD, Eisenhauer PB, Fine RE and Wells JM. Identification of the Protein Disulfide Isomerase Family Member PDIp in Experimental Parkinson's Disease and Lewy Body Pathology. Brain Research. In press.

Carreras I, Garrett-Young R, Ullman MD, Eisenhauer PB, Fine RE, Wells JM, Conn KJ. Upregulation of clusterin/apolipoprotein J in Lactacystin-treated SH-SY5Y Cells. J. Neurosci. Res. Submitted.

Abstracts

Conn KJ, Garrett-Young R, Ullman MD, Gao WW, Eisenhauer PB, Fine RE, Wells JM. Upregulation of Clusterin/apolipoprotein J in Lactacystin-treated SH-SY5Y Cells. 9th International Conference on Alzheimer's Disease and Related Disorders, July 17-22, 2004

Conn KJ, Carreras I, Ullman MD, Eisenhauer PB, Fine RE, Wells JM. Upregulation of Growth/Differentiation Factor-15 (GDF-15)/Macrophage Inflammatory Peptide-1 (MIC-1) by MPP^+ and Lactacystin in differentiated SH-SY5Y Cells. Society for Neuroscience, 34th Meeting, San Diego, CA 2004

Presentations

Ullman MD. Neuroprotective Ganglisode Derivatives: An Overview. Department of Health and Environmental Sciences, University of Massachusetts Lowell, Lowell, MA. April 21, 2004

CONCLUSIONS

Our experience in synthesizing and developing HPLC procedures to purify semisynthetic ganglioside derivatives provides the opportunity to find improved cytoprotective ganglioside derivatives, to define required functional groups for cytoprotection, and to initiate studies to determine cytoprotective mechanisms. Regrettably, the studies have been impeded by the unfortunate and unavoidable loss of the lysoGM1 supply. Still, progress has been made in the *in vitro* studies. GM1, to approximately 2uM) partially protected SH-SY5Y neuroblastoma cells from MPP^+ toxicity. GM1 concentrations above 2uM appeared to make the cells more vulnerable to MPP^+ toxicity and this phenomenon warrants further investigation in another study. Preincubation of the GM1 was not required. Ganglioside derivatives provide comparable cytoprotection but at lower concentrations than that for the parent compound. These compounds were to be tested in the endothelial cell model of the BBB. It

was found that MS and MS^n could be used to detect ganglioside derivatives that crossed the *in vitro* BBB model.

Experiments were performed to further establish the mechanism of MPP^+ toxicity, to help in the delineation of mechanisms by which ganglioside derivatives are cytoprotective. Experimental modeling of Parkinson's disease neurodegeneration using MPTP and MPP^+ identified changes in gene expression of different endoplasmic reticulum stress proteins associated with MPTP- and Parkinson's disease-related neurodegeneration. The protein disulfide isomerase (PDI) family member pancreatic protein disulfide isomerase (PDIp) was found to be uniquely upregulated among PDI family members following exposure of retinoic acid-differentiated SH-SY5Y cells to MPP^+ or to the proteasomal inhibitor lactacystin. Further, lactacystin treatment increased clusterin expression. The development of familial and sporadic Parkinson's disease has been associated with the ubiquitin-proteasome system dysfunction and aberrant protein degradation. These findings on altered gene expression in experimental models of Parkinson's disease emphasize the importance of understanding the mechanisms of MPP^+ toxicity in attempts to further define mechanisms of ganglioside neuroprotection because by better understanding neurotoxic and neuroprotective mechanisms, specific steps in the cell death process can be targeted.

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